

Alpha-synuclein, whose amyloid aggregates are a hallmark of Parkinson's disease, has been suggested to have an interaction with biological membranes that is crucial in synaptic endocytosis. Alpha-synuclein changes the conformation to alpha-helix upon binding to membranes, however, little is known about the molecular mechanisms of this interaction. We studied vesicle tubulation by alpha-synuclein, presumably induced by its amphipathic helix wedging membranes, thereby altering their curvature. We investigated its structural profile and found that alpha-synuclein transforms vesicles into tubular micelles and further into lipid particles, concomitantly with the synuclein conformational change.

Endophilin is a member of the BAR superfamily. The current view is that it recognizes a nascent endocytic vesicle protruding from the synapse by its crescent shape. Its binding then alters the membrane curvature, leading to neck formation and the recruitment of dynamin to this site. On exposing vesicles to endophilin, we observed the formation of tubes varying in diameter from 70 Å to 400 Å, with the thinnest tubes to be a protein-coated tubular micelle. The degree of the squeezing depends on the local density of endophilin on the membrane.

1060-Symp

Using Two-Dimensional Crystals of Aquaporin-0 to Investigate Lipid-Protein Interactions

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Membrane proteins play crucial roles in many cellular processes such as signaling, nutrient uptake and cell adhesion. Although the lipid bilayer influences many aspects of membrane protein function, our understanding of lipid-protein interactions is limited. Aquaporin-0 (AQP0) is a water channel in the lens membrane, but it also assembles into orthogonal arrays that form membrane junctions between lens fiber cells. *In vitro* reconstitution of AQP0 with the lipid dimyristoyl phosphatidylcholine (DMPC) yielded large and well ordered double-layered two-dimensional (2D) crystals that allowed electron crystallographic structure determination of AQP0 and its surrounding DMPC bilayer at 1.9 Å resolution. Since AQP0 forms high-quality 2D crystals not only with DMPC but also with various other lipids, AQP0 2D crystals are an ideal model system to investigate lipid-protein interactions. By studying AQP0 2D crystals formed with different lipids, we can begin to address very basic questions in membrane biology, such as the driving forces that define lipid-protein interactions, the effects of hydrophobic mismatch, and the molecular basis of raft formation.

Platform: Proton and Ligand-gated Ion Channels

1061-Plat

pH-Dependent Conformational Changes of the Voltage-Gated Proton Channel Recorded with Patch Clamp Fluorometry

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The voltage gated proton channel Hv1 has a remarkable architecture. It contains only a voltage sensing domain (VSD, homologous to VSDs of classic tetrameric voltage gated cation channels like Shaker), but lacks a traditional pore domain. Recent studies identified key residues that determine selectivity and located crucial parts of the pore in the VSD. Hv1 is gated by voltage and also by the transmembrane pH gradient. While the fourth transmembrane segment (S4) has been shown to undergo a voltage-dependent motion, suggesting a role in voltage sensing, as in classical VSDs, the protein motions associated with pH sensing are not understood. Here, we use the technique of Patch Clamp Fluorometry to investigate conformational changes of ciona Hv1 in response to changes of internal pH. We labeled the channel at an external site of S4 with the environmentally sensitive fluorophore 2-((5(6)-Tetramethyl-rhodamine)carboxylamino)ethyl Methanethiosulfonate (MTS-TAMRA) and compared the fluorescence signal at different internal pHs and voltages in real-time. Changing internal pH can induce a conformational change visible at S4 in the resting state, without opening of the channel. This indicates that pH gradient sensing does not require a current flux through the channel's pore.

1062-Plat

Molecular Determinants of Hv1 Inhibition by Guanidine Derivatives

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The voltage-gated proton channel Hv1 is known to play important roles in proton extrusion, pH homeostasis, and production of reactive oxygen species in a variety of cell types. It has been recently implicated in cancer development and neuronal death during ischemic stroke. The channel is a dimer made of two voltage sensing domains (VSDs) each containing a gated proton perme-

ation pathway. We have identified guanidine derivatives that inhibit the Hv1 VSDs with a mechanism similar to pore block by quaternary ammonium compounds in sodium and potassium channels. Each VSD in the dimer has its own binding site facing the inner side of the membrane and accessible only in the open state. As long as the inhibitor is bound, the gate in the VSD cannot close. In addition, inhibitor unbinding from one VSD is controlled by the neighboring VSD via tight allosteric coupling between gates. Here we measure the inhibition of different Hv1 mutants produced by structurally related compounds and use a thermodynamic cycle analysis approach to identify molecular characteristics of the channel and guanidine derivatives which are important for tight binding. Understanding how compounds like guanidine derivatives interact with the Hv1 VSD and block proton conduction is an important step toward the development of pharmacological treatments for diseases caused by Hv1 hyperactivity. This work is supported by NIH (grant GM098973) and by the American Heart Association (grant 09BGIA2160044).

1063-Plat

Cooperative Opening of Voltage Gated Proton Channels Involves Inter-Subunit Interactions

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Voltage-gated proton (Hv) channels have two subunits. Each subunit has a permeation pathway, but opening of the two pathways is highly cooperative. However, Hv channels lack a classic pore domain. Therefore, it is unclear how Hv channels open their permeation pathways. Using Voltage Clamp Fluorometry (VCF), we detect two fluorescence changes for a fluorophore attached close to the voltage sensor S4 in Hv channels, implying two conformational changes of S4. The first fluorescence change is highly voltage dependent and precedes channel opening, consistent with reporting on independent S4 charge movements in the two subunits in an Hv dimer. The second fluorescence change is less voltage dependent and closely correlates with channel opening and closing. Prevention of dimerization or mutations at the inter-subunit interface alters both the second fluorescence change and channel opening. Our results suggest that, following an initial S4 charge movement in the two subunits, channel opening in Hv channels is due to a second conformational change of S4 involving interactions between the two Hv subunits.

1064-Plat

Voltage Sensing in Hv1 Proton Channels

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Activation of the intrinsic aqueous water-wire proton conductance (G_{AQ}) in Hv1 channels is controlled by changes in membrane potential and the transmembrane pH gradient (ΔpH). The mechanism by which changes in ΔpH affect the apparent voltage dependence of G_{AQ} activation is not well understood. Recent work from our laboratory demonstrates that H^+ current activation is rate-limited by a voltage-independent but ΔpH -sensitive transition when $pH_O \leq pH_I$. In contrast, the Cole-Moore activation delay in Hv1 is relatively insensitive to changes in ΔpH , indicating that early steps in voltage sensor (VS) activation are distinct from later channel-opening transitions. In order to measure initial Hv1 voltage sensor activation in greater detail, we mutated a conserved Arg residue in the fourth helical segment (S4) to His and measured H^+ currents under whole-cell voltage clamp in transfected HEK cells. Consistent with a previous results in the Shaker K^+ channel mutant R362H (Starace and Bezanilla, 2004), we find that Hv1 R205H mediates a robust resting-state H^+ 'shuttle' conductance (G_{SH}) at negative membrane potentials. The midpoint of the G_{SH} -V relation is >100 mV more negative than G_{AQ} -V and G_{SH} gating kinetics are significantly faster than G_{AQ} . Intriguingly, changes in ΔpH that are sufficient to produce a $\sim +40$ mV G_{AQ} -V shift cause G_{SH} -V to shift only $\sim +10$ mV. G_{SH} gating thus reports a conformational change in Hv1 that occurs prior to the opening of G_{AQ} and is differentially sensitive to changes in ΔpH . The data are consistent with the hypothesis that initial gating charge movement occurs early in the Hv1 activation pathway and is thermodynamically separable from aqueous H^+ channel opening.

1065-Plat

Propofol Induced Structural Changes in the TMD of the Prokaryotic pLGIC GLIC

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General anesthesia, a routine and indispensable tool in modern surgery still remains a poorly understood phenomenon. Recent studies suggest that general anesthetics (GAs) mediate many of their anesthetic actions by binding to intra- or inter-subunit transmembrane domain (TMD) cavities of pentameric ligand